MULTIPARAMETRIC IMMUNOASSAYS IN BLOOD FOR THE DIAGNOSIS/SCREENING OF COLORECTAL CANCER (CRC): ACTUAL RESULTS AND FUTURE DEVELOPMENTS

J.P. Charrier, C. Beaulieu, Y. Ataman-Önal, T. Fortin, S. Pons, H. Haïdous, J. Deleforge, G. Choquet-Kastylevsky
Biomarker Research & Validation Department, Clinical Affairs Department, bioMérieux SA, Marcy l'Etoile, France

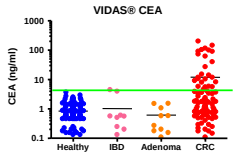
SUMMARY

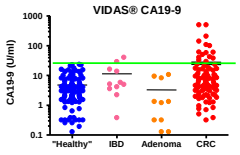
From the 60 proteins identified in colorectal tumor and selected for evaluation in patients blood samples, 16 assays were constructed or probed using either VIDAS®, or microtiter plate ELISA. Each protein assay was used to quantified a train set of up to 200 sera of colorectal cancer patients (CRC), Inflammatory Bowel Disease (IBD), colonic adenoma or "healthy" controls.
Results for each assay were compared to already known ELISA dosages for markers such as CEA, CA19-9, B2M or TIMP-1.
The assays showing the higher discrimination between colorectal cancer and healthy controls were subsequently combined in a multiparametric assay to select the more advantageous assays. These assays will be ultimately tested on a validation cohort of up to 1000 patients (ongoing INCA collection of individuals screened for CRC).
Using 3 to 10 tests combined in multiparametric assays, sensitivity and specificity were simulated on the training set. Results are displayed for 11 biomarkers and the multiparametric assays.
The performance of the multiparametric assays, with only 16/60 new markers developed as assays, seems to be in agreement with the further requirement of a mass-screening assay.

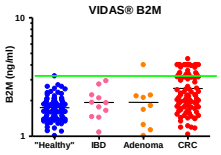
RESULTS

MONOPARAMETRIC ASSAY ALGORITHM

Biomarkers available in the bioMérieux menu:



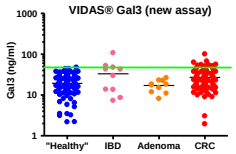


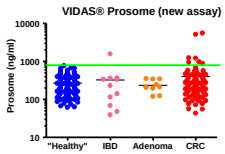


VIDAS System

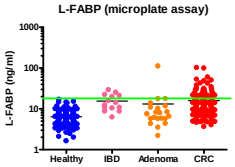
The VIDAS test system uses a single-dose, ready-to-use reagent.
It uses the ELFA assay principle, combining the ELISA test method with a final blue fluorescent reading.
This technology ensures excellent result sensitivity and specificity.

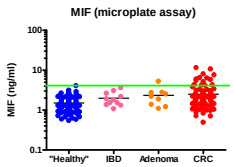
Example of new biomarkers developed on the VIDAS system:

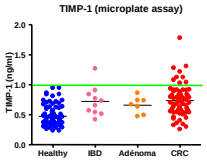




Example of new biomarkers developed on microtiter plate:



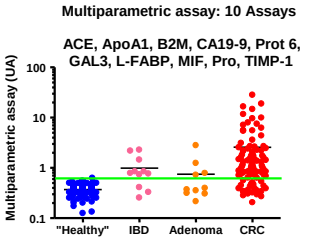


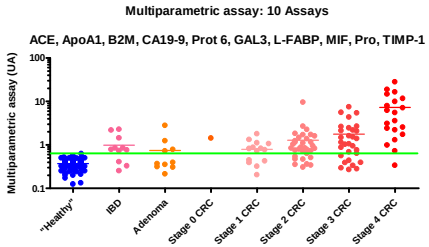


MULTIPARAMETRIC ASSAY ALGORITHM

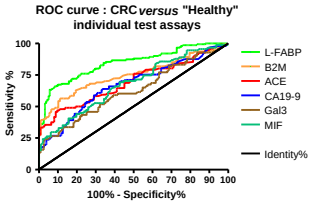
1 - Cut-off for each monotest assay: defined by default as the maximal value of the "healthy controls".
2 - Normalization of each assay value dividing by the cut-off.
3 - Weighting of value >1 using a unique multiplication factor (3 by default).
4 - Mean of all assay values for each patient (multiparametric assay).
5 - Cut-off choice for the multiparametric assay: defined by default as the maximal value of the "healthy controls".

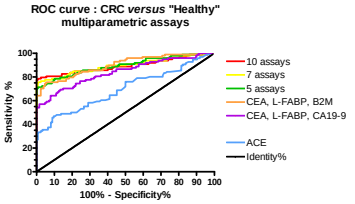
Association of biomarkers for a multiparametric assay:





ROC curves & statistical analysis:





Assay	Area under the ROC curve	95% confidence interval of the area under the ROC curve	P value for difference "healthy" versus CCR	Significance	"Healthy" control	CCR patients
ACE	0,6947	0.6221 to 0.7673	< 0.0001	Yes	124	96
ApoA1	0,8463	0.7333 to 0.9592	< 0.0001	Yes	27	20
CA19-9	0,6701	0.5975 to 0.7427	< 0.0001	Yes	127	97
B2M	0,7475	0.6769 to 0.8181	< 0.0001	Yes	108	94
GAL3	0,6236	0.5430 to 0.7042	0,003032	Yes	115	83
HSP60	0,612	0.5365 to 0.6875	0,004772	Yes	118	97
L-FABP	0,8446	0.8007 to 0.8884	< 0.0001	Yes	158	150
MIF	0,6773	0.6011 to 0.7534	< 0.0001	Yes	96	91
Prosome (Pro)	0,5173	0.4393 to 0.5952	0,6606	No	122	97
TIMP-1	0,8044	0.7319 to 0.8768	< 0.0001	Yes	80	66
Multiparametric assay - 10 assays	0,8945	0.8455 to 0.9435	< 0.0001	Yes	127	98
Multiparametric assay - 7 assays	0,8968	0.8503 to 0.9433	< 0.0001	Yes	127	98
Multiparametric assay - 5 assays	0,8951	0.8498 to 0.9405	< 0.0001	Yes	127	98
CEA + L-FABP + CA19-9	0,9007	0.8589 to 0.9426	< 0.0001	Yes	127	98
CEA + L-FABP + B2M	0,8405	0.7859 to 0.8952	< 0.0001	Yes	127	98
CEA + B2M + CA19-9	0,8105	0.7511 to 0.8700	< 0.0001	Yes	127	98

Sensitivity / Specificity simulation:

Assays	Cut-off	Sensitivity (%)	Specificity (%)
Multiparametric assay - 10 assays	Default	77,50	100
Multiparametric assay - 10 assays	Tune 1	79,59	97,64
Multiparametric assay - 10 assays	Tune 2	82,65	90,55
Multiparametric assay - 7 assays	Default	71,43	100
Multiparametric assay - 5 assays	Default	61,22	100
CEA + L-FABP + CA19-9	Default	54,08	100
CEA + L-FABP + B2M	Default	64,29	100
CEA + B2M + CA19-9	Default	55,10	100

CONCLUSION

Using proteomic approaches, we were able to discover new protein biomarkers with a potential interest as colorectal cancer diagnostic tools. Mass screening of CRC using blood samples would be a big improvement for CRC management, as it would enhance the compliance to screening programs, thus offering the possibility of easy and efficient mass screening at a cost acceptable for public health authorities.
In this study, we have shown together the feasibility and achievability of such a goal. A first set of candidate biomarkers was evaluated on a cohort of cancer and healthy patients, and the observed performances are aligned with the requirements of CRC mass screening. Studies are ongoing to complete the evaluation of the remaining biomarkers, allowing the choice of the best combination in term of sensitivity, specificity and robustness.